PEGGY GREB (K11340-1)

Plant molecular geneticist Sheila McCormick uses a vacuum to collect pollen from *Arabidopsis* (thale cress) plants genetically engineered in her laboratory.

Probing the Puzzles of Plants' Pollen and Eggs

Achoo!

Welcome to spring, when flowers burst into bloom—and pollen counts soar. Those soft, crumbly grains of pollen that leave many of us sneezing and red-eyed are, as we all learned in grade school, essential for fertilizing flowers so that they will form many of the foods we eat and enjoy—plump, juicy tomatoes or golden ears of sweet corn, for example.

But those early lessons might not have covered some of the more complicated steps that are part of the carefully orchestrated fertilization process of our planet's flowering green plants. "Floral fertilization," explains ARS plant molecular geneticist Sheila M. McCormick, "occurs deep inside the flower when the two male sex cells that are carried in each pollen grain meet, recognize, and fuse with two different cells in the female part of the flower—the embryo sac. One embryo sac cell is the egg, just like in animals. But the other—the central cell—is unique to plants."

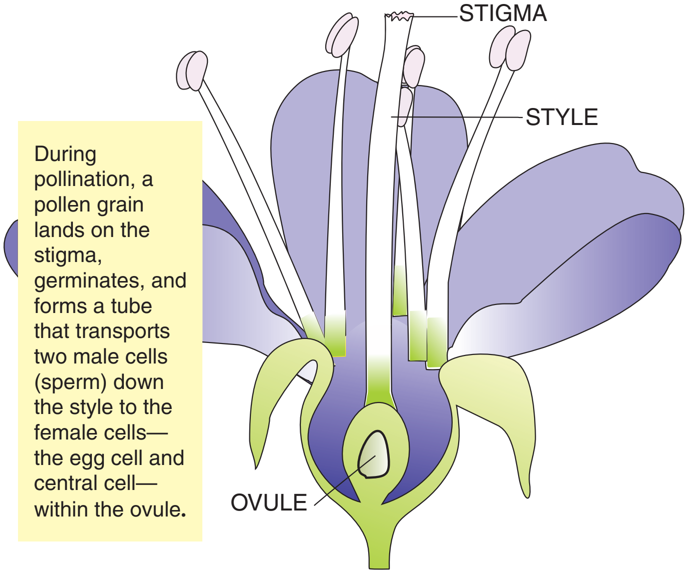
This fertilization of the two female cells by the two male cells, called double fertilization, is critical to creating the crops that keep us healthy and well fed. For most of Earth's flowering plants, no fusion means no crop.

Equally important are the steps that lead to that fusion, such as the sprouting and elongating of the pollen tube. The slender tube forms when the pollen grain lands on the top of the flower's columnlike style, then swells with water. As it extends, the tube transports its passengers, the male sex cells, down through the style, delivering them to the female cells—where fertilization occurs.

What Roles Do Genes Play?

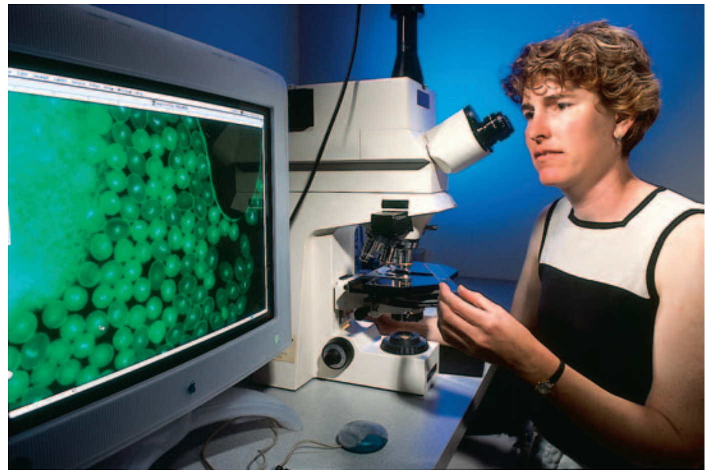
Some of these facts about floral fertilization have been known for at least a century. But many of the details still remain a mystery. In particular, researchers know very little about the genes and the products of those genes—proteins—that are key players or at least play important supporting roles in the fertilization drama.

Why do we need to know more? Scientists would like to be able to alter the activity of genes that block fertilization of certain wild species with their domesticated cousins, for example. Until such barriers are overcome, wild relatives' prized traits cannot, in many cases, be easily moved into their cultivated



During pollination, a pollen grain lands on the stigma, germinates, and forms a tube that transports two male cells (sperm) down the style to the female cells—the egg cell and central cell—within the ovule.

PEGGY GREB (K11342-1)



Michele Engel, postdoctoral fellow, observes *Arabidopsis* pollen with a microscope. The plants were altered so that the male sex cells (sperm) appear as glowing green dots within each pollen grain.

kin. The sought-after genes might enable tomorrow's plants to thrive with less fertilizer, less water, or perhaps even less pesticide.

McCormick and colleagues are uncovering new facts about infrequently researched interactions of plants' male and female cells. She's with the Plant Gene Expression Center at Albany, California, which is staffed by scientists from ARS and the University of California, Berkeley.

The Albany group is identifying key genes, sleuthing their exact roles in successful fertilization, and sharing these discoveries with researchers worldwide by reporting the findings in leading scientific journals and by posting the sequences of newfound genes on the publicly available GenBank genome database (www.ncbi.nlm.nih.gov).

Cells Send Secret Messages

The researchers are deciphering some cell-to-cell communication in which protein molecules send messages to other protein molecules. In one aspect of this work, the scientists are investigating how such messages may help the growing pollen tube find its way to the embryo sac.

Two types of protein molecules, known as ligands and receptor kinases, may assist. Both kinds of molecules are formed according to instructions carried by genes and are secreted from cells. "Receptor kinases are already known to bind to ligands," says McCormick. "The binding of these proteins sends a signal. If this same scheme operates in pollen tubes, such signaling might help guide the pollen tube."

To learn more, the researchers experimented with receptor kinases that they had discovered earlier in studies of tomato pollen. The kinases, which they had named LePRK1 and LePRK2 for tomato's scientific moniker, *Lycopersicon esculentum,* served as the lures or baits for partner molecules—the ligands—that might be secreted from cells in the female part of the flower. "We identified many potential ligands," McCormick reports.

Fusion: Finding the Right Partner

Once the two male cells come into the vicinity of the egg and central cell, how do they know which cell is their correct fusion partner? Perhaps signaling molecules, similar to those that may assist the pollen tube's journey, exist on the female and male cells, McCormick says. To find out, her team is determining the makeup, or DNA sequence, of genes that are switched on or off in the male cells and in the embryo sac cells. This is a first step toward learning which proteins are encoded by those genes and whether those proteins act as signaling molecules on the surfaces of these cells.

So far, McCormick and co-researchers have discovered hundreds of new genes in these cells. (There's more information about this research on the World Wide Web at www.pgec.usda.gov/McCormick/McCormick/mclab.html.)

Their probing into the puzzle of plants' pollen and eggs has won funding from the ARS national postdoctoral fellowship program (see *Forum*, page 2) and $1.1 million from the National Science Foundation. The pioneering investigations will likely yield even more clues about the 144-million-year-old reproductive ballet of the world's flowering green plants.—By **Marcia Wood,** ARS.

This research is part of Plant Biological and Molecular Processes, an ARS National Program (#302) described on the World Wide Web at www.nps.ars.usda.gov.

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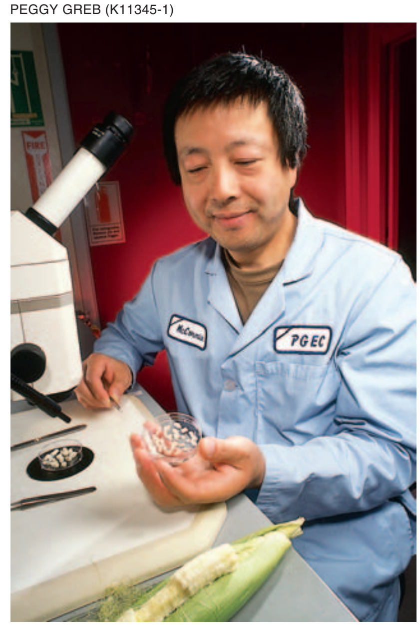

To learn more about genes involved in fertilization, Heping Yang, postdoctoral fellow, prepares to dissect embryo sacs from within corn kernel ovules.